

FIG. 1

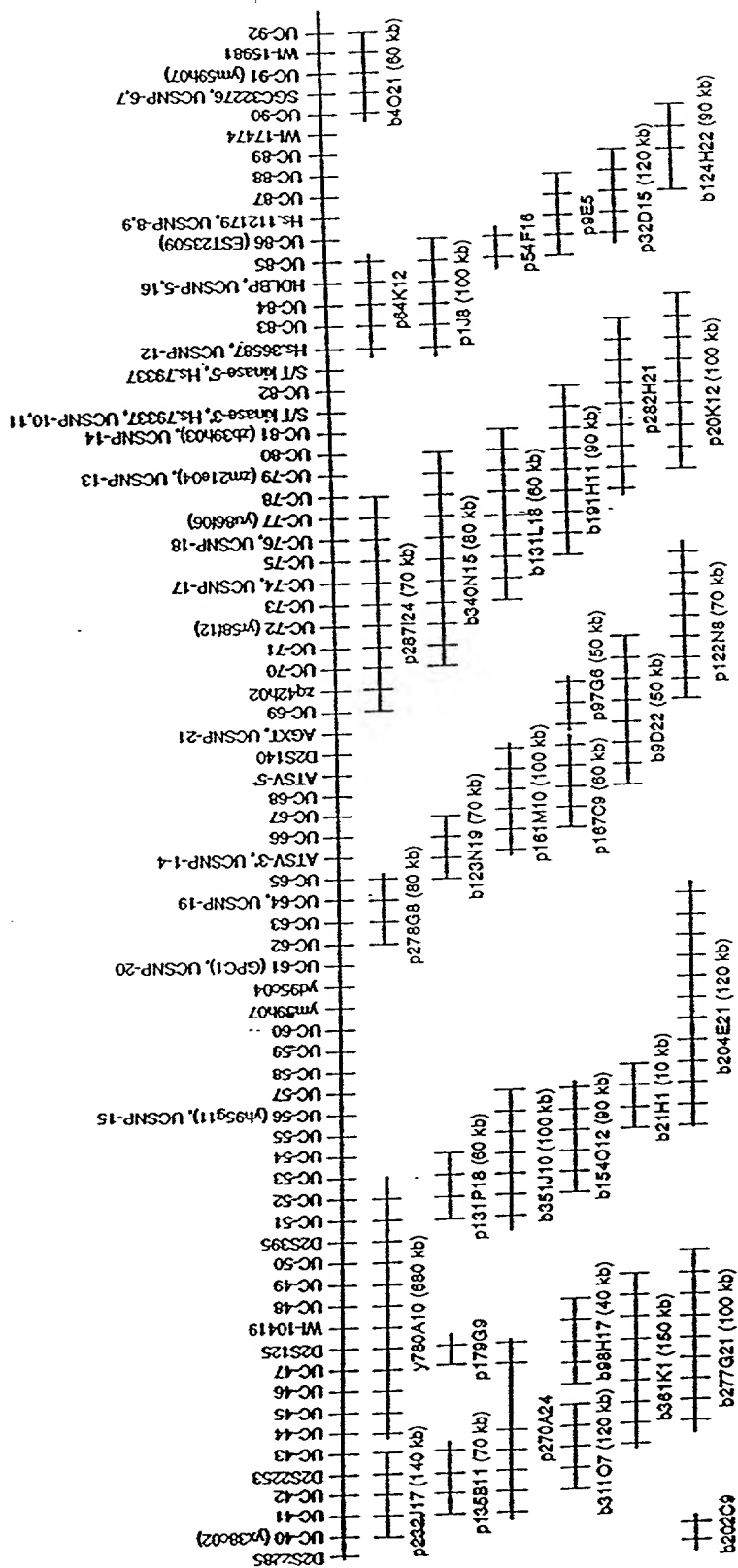


FIG. 2

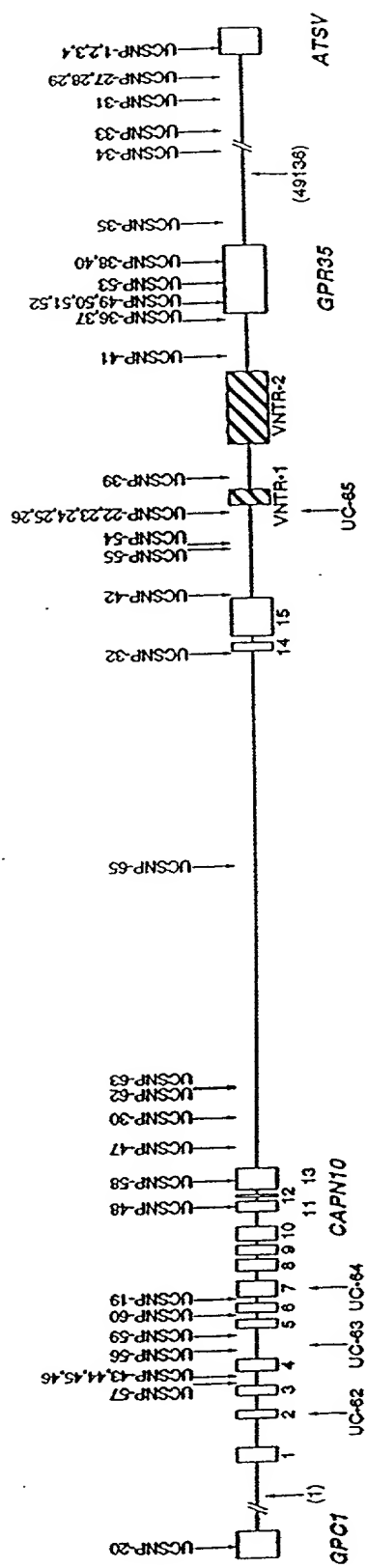


FIG. 3

TO 2730" 22889260

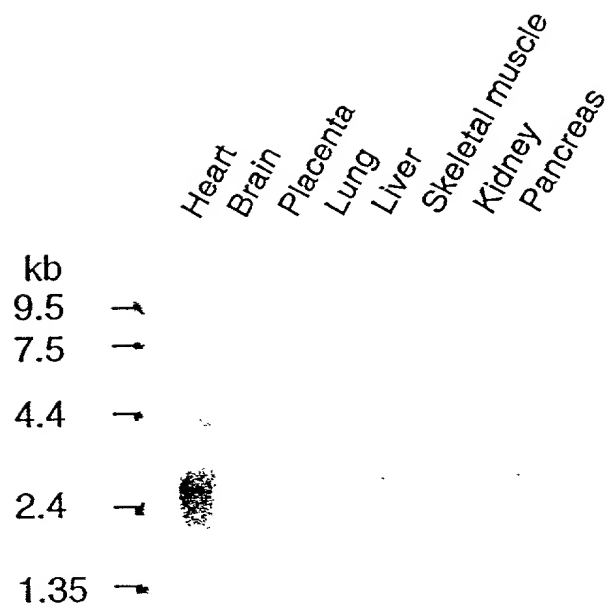


FIG. 4

	Domain I	Domain II
hCAPN5	-----HFCSCVPTEDQNYSAHQDQRRKRVLFEDPLFPAIDDSLY--KCTGPE-----AVMKRKPCTGCEDEPRFVDC	67
mCAPN6	-----HGPPLKLFNQKYQELQKQCKEDGRLECDPTFLPNDSLFNRLLPG-----KVMKRPQDISDOPHILVGN	67
hCAPN3	MPITVISASVAPRTAAEPSPGCPVPHPAQSKATEAGGNGPSGIYSALISRNPLIGVKEKTYDQAKKCKEKLVLVDPEFPDCTSLFYSQKFF-----IQVWKRPPEICEKNERFIIDG	115
hCAPN9	MPITY-----RAPCPQAPVP-----KDARITHSSGQSFQMRQECLEQRTLEDAEPFASNSSLFYSERFQ-----IPFWKRPGEIVKNEPFILOG	83
hCAPN1	-----HSEELITFVYCTGVSAQVQKQKARELGIG-----RHENATKYLQDQYDQLRVECLQSGTLFRDEAFPPVPQSLGQDAGNNSKKTGLMKRPEITELLSPNQFTVDC	101
hCAPN2	-----KAGIAAKLAKDREAAGLG-----SHERATKYLQDQYDQLRVECLQSGTLFRDEAFPPVPQSLGQDAGNNSKKTGLMKRPEITELLSPNQFTVDC	91
rCAPN8	-----HAAIAAGVSKQRAVABLG-----SMQNAVYKLGQDQYDQLRVECLQSGTLFRDEAFPPVPQSLGQDAGNNSKKTGLMKRPEITELLSPNQFTVDC	91
hCAPN10	-----HRAGRGATPARELFDAFAFPADSSLFCDLSTPLAQFREDITMRPEICATPRNFPD	59
hCAPN5	ISSHDLIQQQVGNMVEVAACSSLASRESLAKQVYIPDKWEQEMPRKACAYAGIFHFHFRVLG--HVDVVIDERLPTVNMQLTYCHSNRNEFWALWEKAYAKLAGCCYQALDGGNTADALY	186
mCAPN6	ISNHQLIQGRLGNKAMISAFSCLAQVQSHWTKAIPNKKDQEMDPRKPEKXAGIFHFRHFWGEMTEVVVIDOLLPITNGDLVFSFSTSHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	187
hCAPN3	ANRTDIOGELGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	229
hCAPN9	ATRTDIOGELGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	197
hCAPN1	ATRTDIOGELGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	215
hCAPN2	ATRTDIOGELGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	205
rCAPN8	ATRTDIOGELGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	205
hCAPN10	PRDGVKQGLGDGMCLAAIASLTINQKALRVIP-----HQDSFENYAGIFHFQVRYGEMVVDVVIDOLLPITNKKVFTKSNHNEFWALWEKAYAKLAGCCYQALDGLTITDIIM	175
hCAPN5	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----TAADK-----EARIACGLNKGHAYAVTDV	258
mCAPN6	DFTOGLAEIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----SQEDQ-----EVEIDWGLKAGTYTTHDI	259
hCAPN3	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----APSDMYKIMKAIERGLHGCISIDGTHNYTGTSPGLNAGELTARVNRHNSLQOSDLPDGRSDERPTITLIPVQVTEHACGLNKGHAYAVTDV	341
hCAPN9	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----APENYELKALRGSLGCGIIDT-----RSAAES-----EARTFPGLKNGHAYAVTDV	261
hCAPN1	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----APSDLYQIILKALRGSLGCGISIDT-----S-----VLDE-----EATTFKGLNKGHAYAVTDV	279
hCAPN2	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----PPNLFKILKALRGSLGCGISIDT-----S-----AAS-----EATTFKGLNKGHAYAVTDV	269
rCAPN8	DFTOGVSEPIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----PPNLYTIQKALRGSLGCGISIDT-----T-----AAEA-----EATTFKGLNKGHAYAVTDV	269
hCAPN10	DFTOGLAEIDLTGDFANDETKRNQLFERMLKVNHRGGLISASIKAV-----VLS-----PRAGRELGEHAFITVSD	245
	Domain II	Domain III
hCAPN5	RKVLGHGLLAFFSEKLDNIRLRNPNWGERENHNPWSDTSEEMQKYSERERKHGVTVDQGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----HGAMTLHEDPRQNRG	373
mCAPN6	RKLRLGERLNVSTFKLNVRLNPNWGERENHNPWSDTSEEMQKYSERERKHGVTVDQGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----VGCITVDDEPLMRS	371
hCAPN3	DEVPF-----KGEVKILVLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----BGRVNRG-----CSA	444
hCAPN9	DQVSE-----RGQRILRLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----QDSVNRG-----STA	364
hCAPN1	KQVNY-----RGQVSLRLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----DQVNRG-----STA	381
hCAPN2	EEVES-----HGSQELRLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----DQVNRG-----STA	371
rCAPN8	EEVNE-----HGRPELRLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----DQVNRG-----STA	371
hCAPN10	RELQG-----QACQCIILLRLRNPNWQVEMNGSWSDRWKDSFVDEKDEKARLHQVTEDEGEFVETEDVCRITFDITIKCRVINTSHL--SIHKTWEARL-----QSA	352
hCAPN5	GGCINRQDITFFNQPYIFEVKPPD-----EVLCIQQPKRSTRBCKGENLAIGDFIYKVE-----E-----NRQYRHESL--QKKAASSIKYNSRSLRDTQDQGRVYIIP	472
mCAPN6	GGCINRQDITFFNQPYIFEVKPPD-----KVINSLQQRDLRTYRMRGRDNVYIGFELEKVE-----H-----NRERLRLYLQERAGSTYITDTRVFLSKLKGSTYVPT	472
hCAPN3	GGCINRQDITFFNQPYIFEVKPPD-----DQDSEV--ICSFVALHQRNRRKRLG--ASLETIGAIYVPEKRRGK--QHLQDQFLYNASTAKRSKITDRKESQRFLPPEVNTVPS	559
hCAPN9	DQVSE-----QGE-----CSFVALHQRNRRKRLG--ANVITIGAIYVPEKRRGK--DQD-----EHLNEDFFRYHSHARSKITDRKESQRFLPPEVNTVPS	471
hCAPN1	GGCINRQDITFFNQPYIFEVKPPD-----GCSFVALHQRNRRRREFG--RDMETIGAIYVPELWQQAVALRDEFLANASRASEQ--INLREVSTREFLPPEVNTVPS	499
hCAPN2	GGCINRQDITFFNQPYIFEVKPPD-----GCTFLVGLIQHRRRRQRNG--EDMTIGFVYVPEELSGQINILHLSNMFFLNARERSDIT--INLREVLRNRLPPEVNTVPS	487
rCAPN8	GGCINRQDITFFNQPYIFEVKPPD-----CCTVLLGLHQRNRRRQRNG--QCHLSIGYAVYQIPKELSHTDARLGRDFFLGRQPSSTYINLREVSSREFLPPEVNTVPS	489
hCAPN10	GGCINR--SGPPSNPKFVLESEPSVYIAVLRQSRLLAANDAGARALVGDSEHSHWSPASIBGKHQAVGLHAKVKEKRVNLRVLSHPPVAGTACHAYDEKRVHLRCELSPGYTLAVPS	471
	Domain III	Domain IV or T
hCAPN5	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----C-----KSSLCQYQLAVTVHVLGAAGLKD-----	530
mCAPN6	MFHQGRITSEFLRIFSEAFVQLRELTLDRPKMS-----C-----KRLARGYFVWVQITVHSAAGLEKLY-----	532
hCAPN3	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	546
hCAPN9	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	572
hCAPN1	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	572
hCAPN2	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	559
rCAPN8	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	562
hCAPN10	TFEPHGTGEFLRVFTDVPNSCRELALDEPMIT-----E-----PKPTP--PDQTEEBQRFNALLFQVWAGDEKVEYTAEEVYLVNA	541
hCAPN5	-----SPGTANSYVIKCEGQKQVSAVQKQ-----TSTPEIRNVKGFIRKRLKSPQITVQVNR-----VLKDEFLQVHLKADPQNLQALHTLHEDNSRQ	618
mCAPN6	-----ANETVNPYLIIKQGEVRSVPVQRN-----TVHATFDTQALFYKRIIDIIIPVQNR-----KFDQFLQVYVTDADSECDRLKSLYLKRGKGP	620
hCAPN3	VVNRKRLKTHGTFLESCRSIALMDTDSGKRLNQEFHILANKIKAMQKLFKHITDQSGTINSTEHNRVAVNDAGFHLARQVYDIITRATADKRRHEDFUSFICCVRLGDFRAFIHAF	795
hCAPN9	VVNRKRLKTHGTFLESCRSIALMDTDSGKRLNQEFHILANKIKAMQKLFKHITDQSGTINSTEHNRVAVNDAGFHLARQVYDIITRATADKRRHEDFUSFICCVRLGDFRAFIHAF	666
hCAPN1	IISSKRLKTHGTFLESCRSIALMDTDSGKRLNQEFHILANKIKAMQKLFKHITDQSGTINSTEHNRVAVNDAGFHLARQVYDIITRATADKRRHEDFUSFICCVRLGDFRAFIHAF	692
hCAPN2	VVNRKRLKTHGTFLESCRSIALMDTDSGKRLNQEFHILANKIKAMQKLFKHITDQSGTINSTEHNRVAVNDAGFHLARQVYDIITRATADKRRHEDFUSFICCVRLGDFRAFIHAF	677
rCAPN8	VVNRKRLKTHGTFLESCRSIALMDTDSGKRLNQEFHILANKIKAMQKLFKHITDQSGTINSTEHNRVAVNDAGFHLARQVYDIITRATADKRRHEDFUSFICCVRLGDFRAFIHAF	687
hCAPN10	-----PCFFSVPGGPRCVRITLHQRKPSD-----TEFPIGRIITQVPEGGESQAPPLILLQEPFLKSCVHRYAGEVSLRCLLPAGTYKYVPSVITLDEGAFYVTIATRIDRPSIHQS	65
hCAPN5	PSNLPGTVAVHILSSTSLNAV--	639
mCAPN6	AKVKQCHISFVLISSDOLTEL--	641
hCAPN3	DRDGGIILKINLEWLGUTHA	821
hCAPN9	STNKEFHLINLEFHLTHN1	690
hCAPN1	DTLDGCVTFDLFKMLQUTHA	714
hCAPN2	DPNTGTFELDLISWLCFSVL--	708
rCAPN8	DRDGGIIVQLSLAEWLCVVL--	703
hCAPN10	EDLQGLQGVSVAVHVKT----	672

Fig. 5

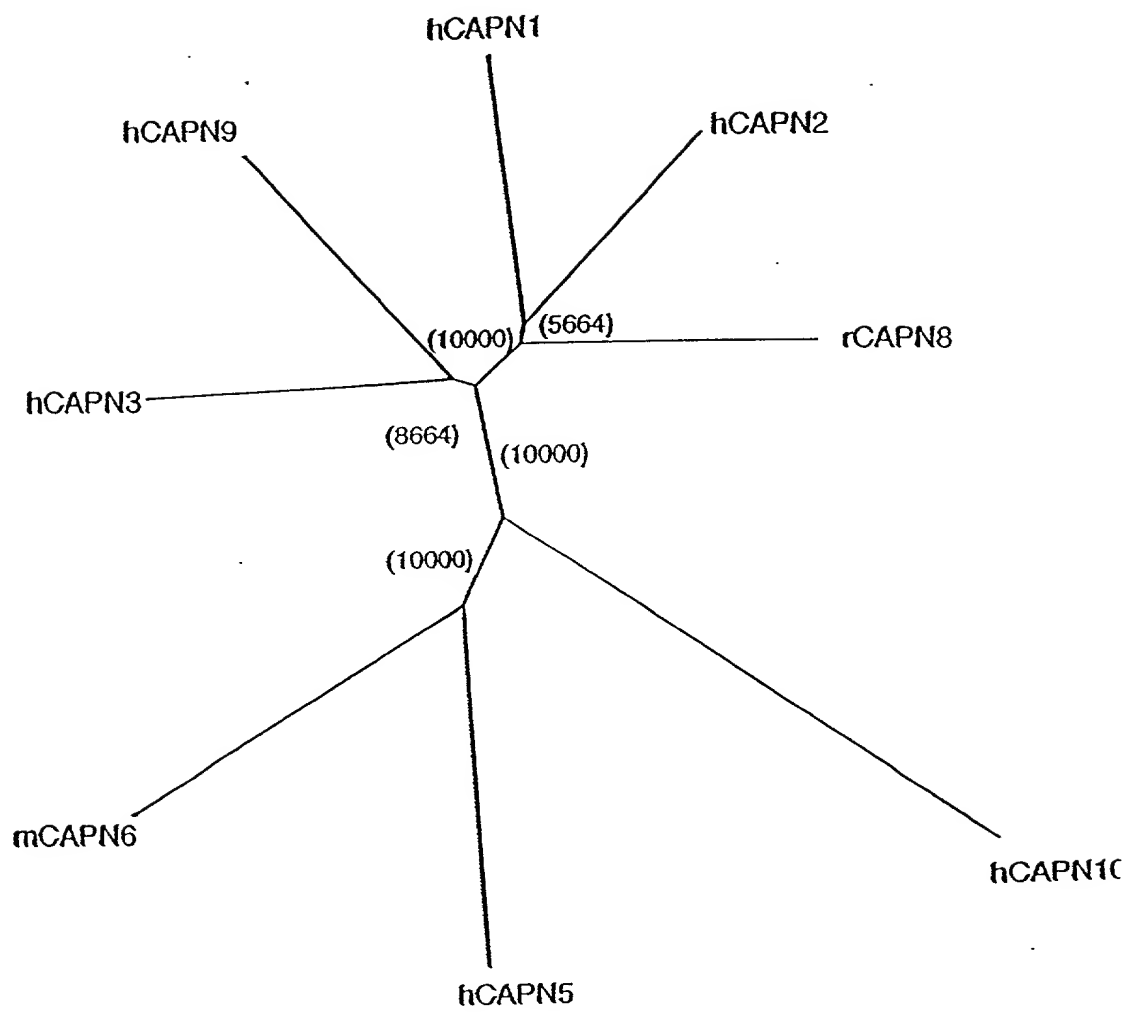


FIG. 6

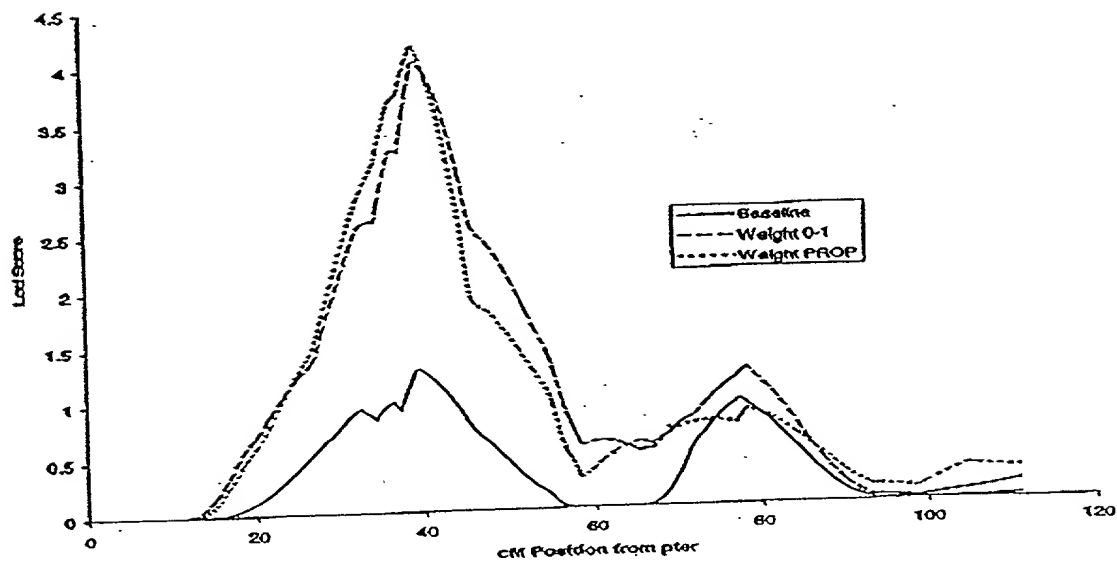


FIG. 7A

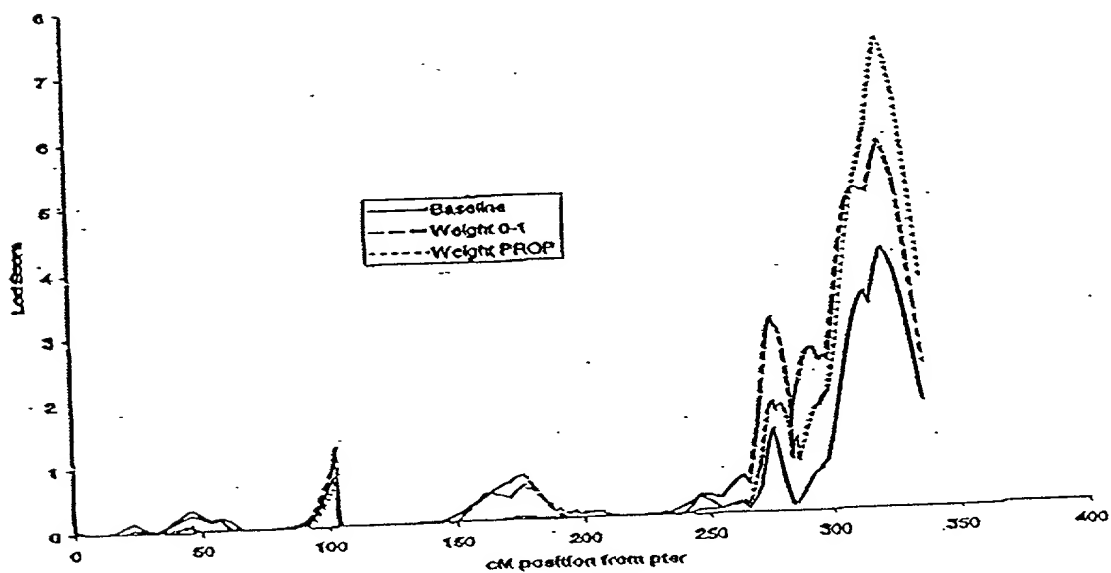


FIG. 7B

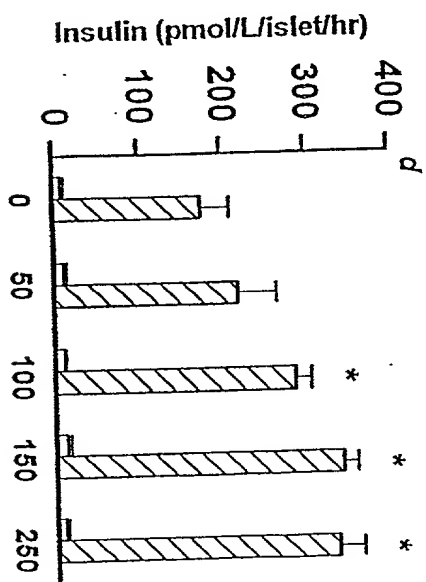
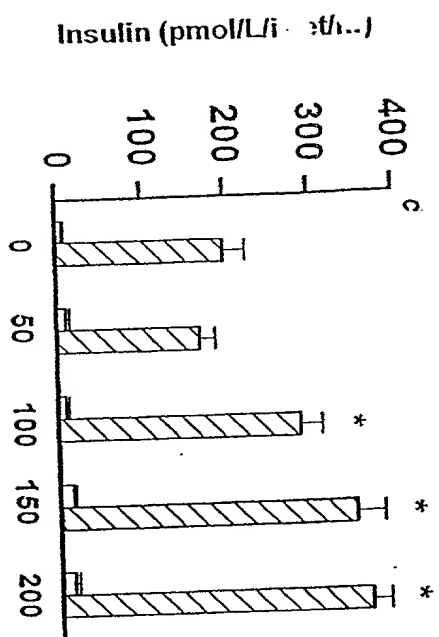
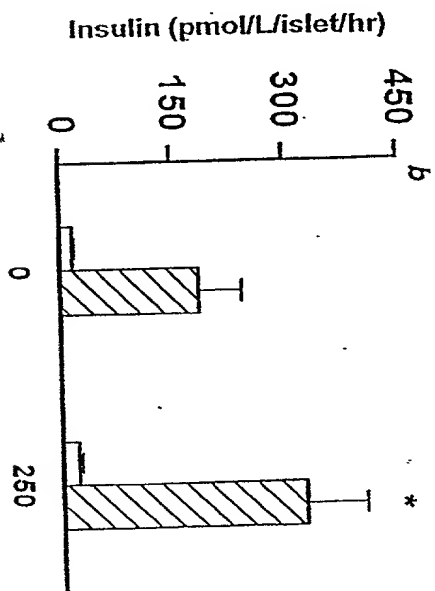
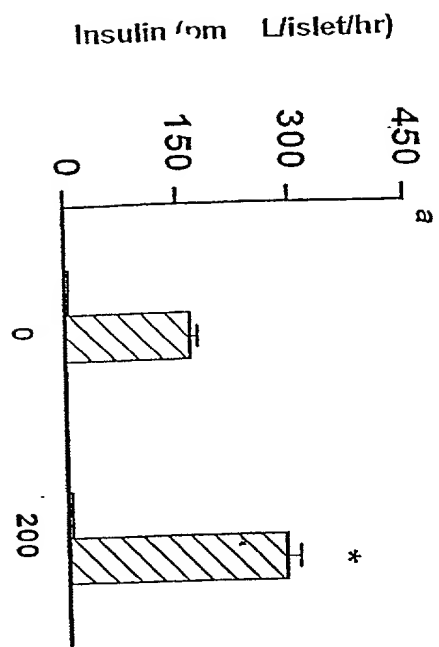


FIG. 8



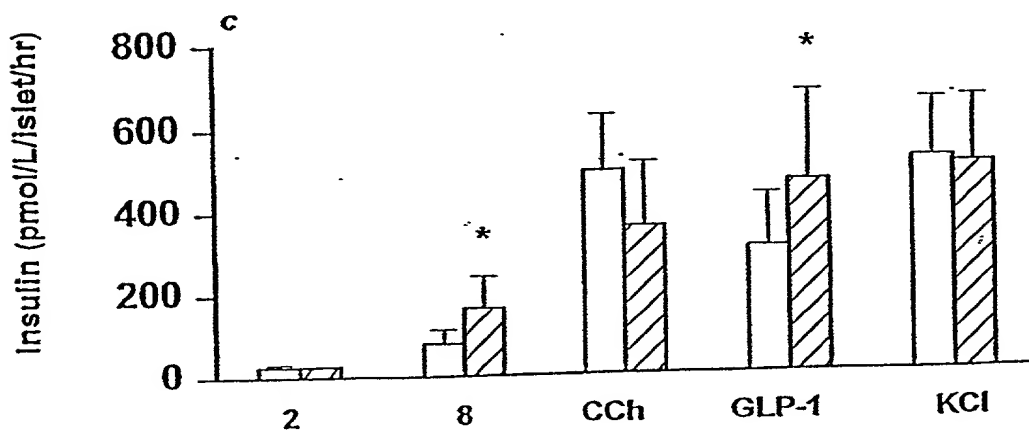
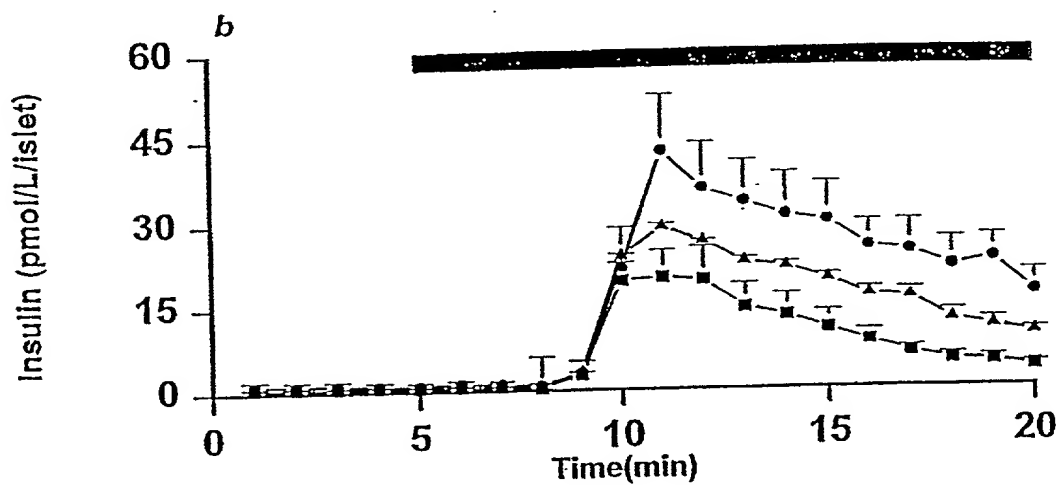
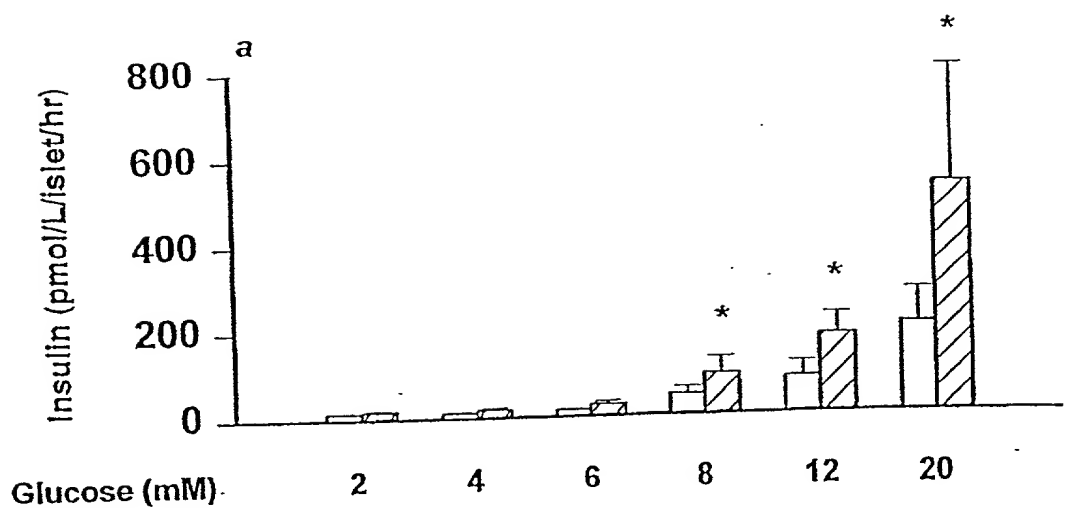


FIG. 9

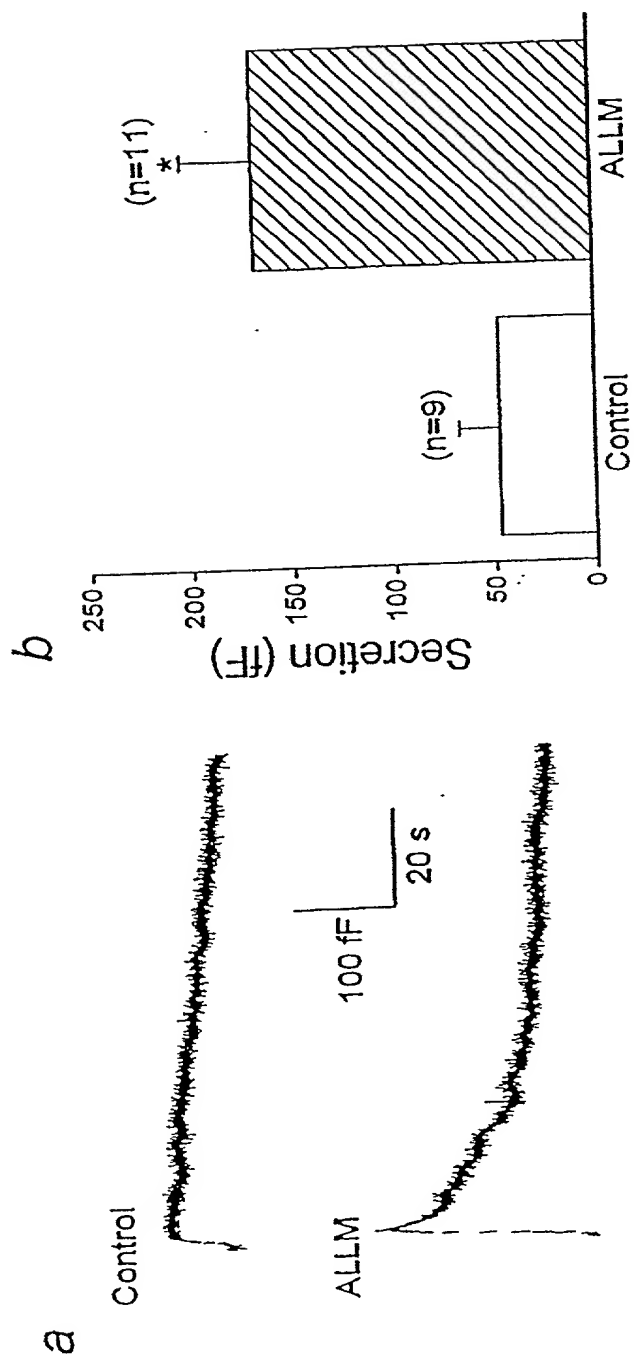


FIG. 10

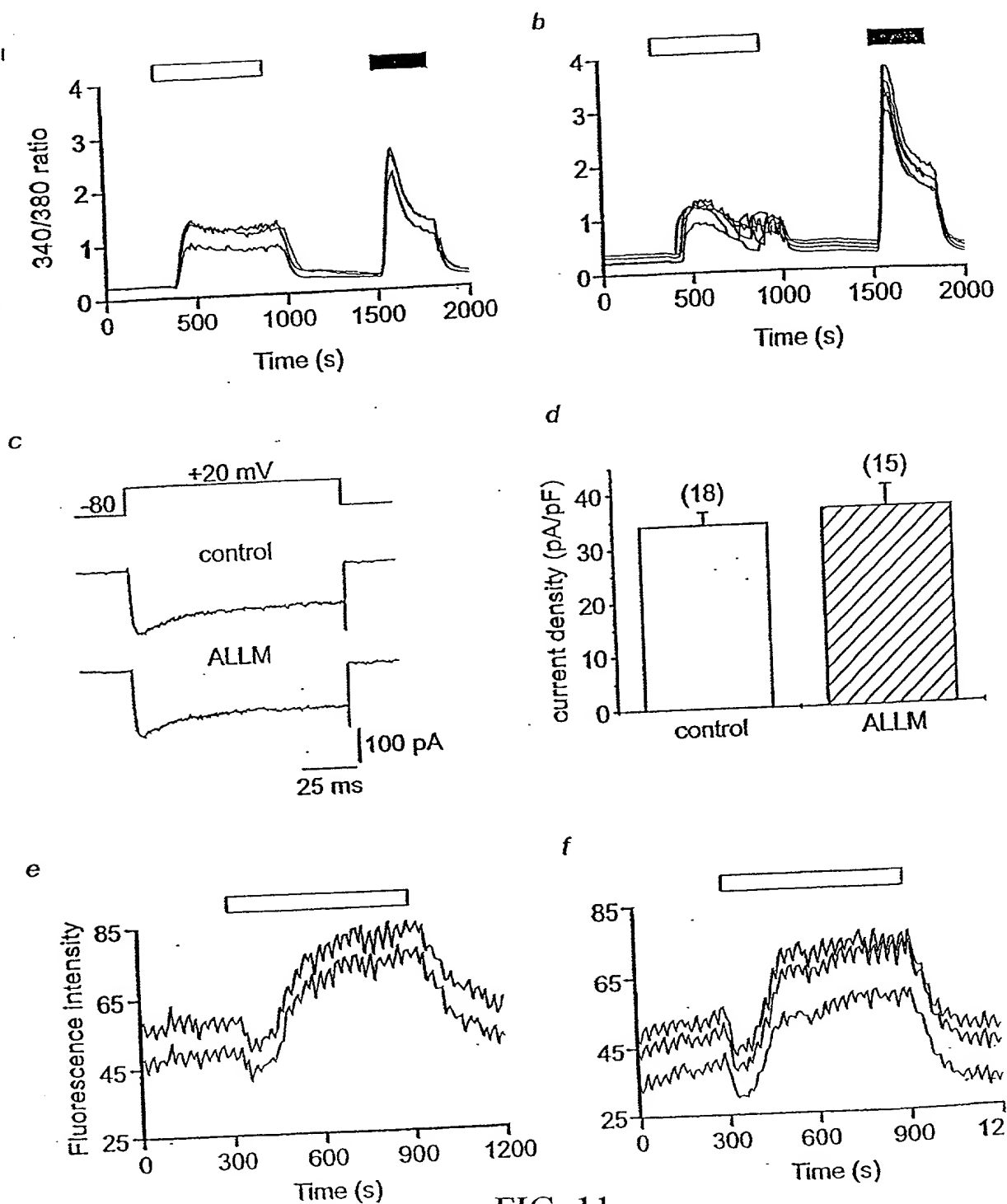


FIG. 11

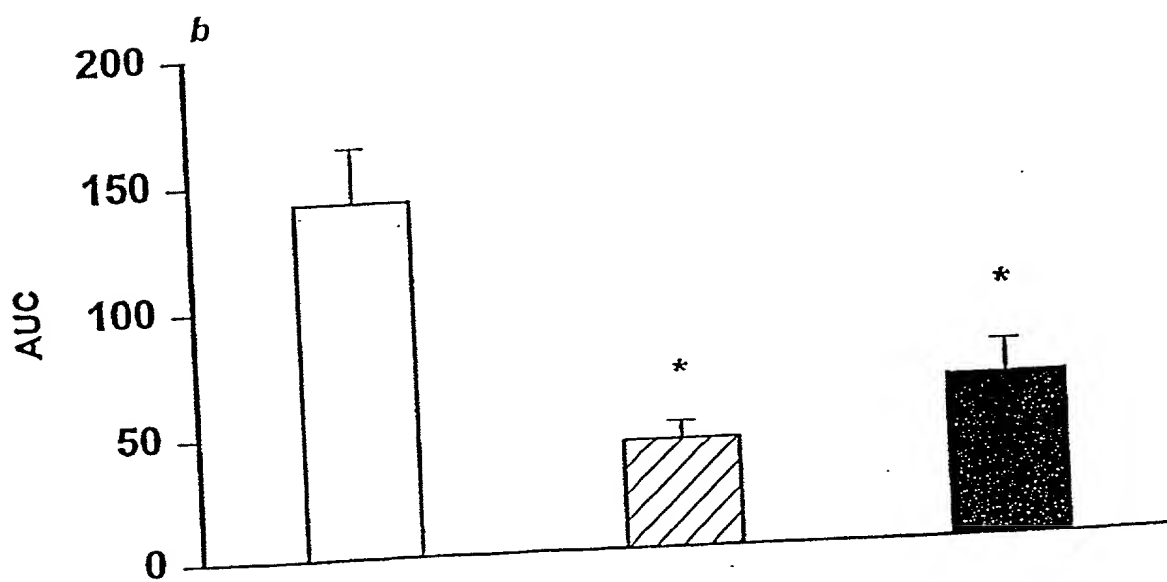
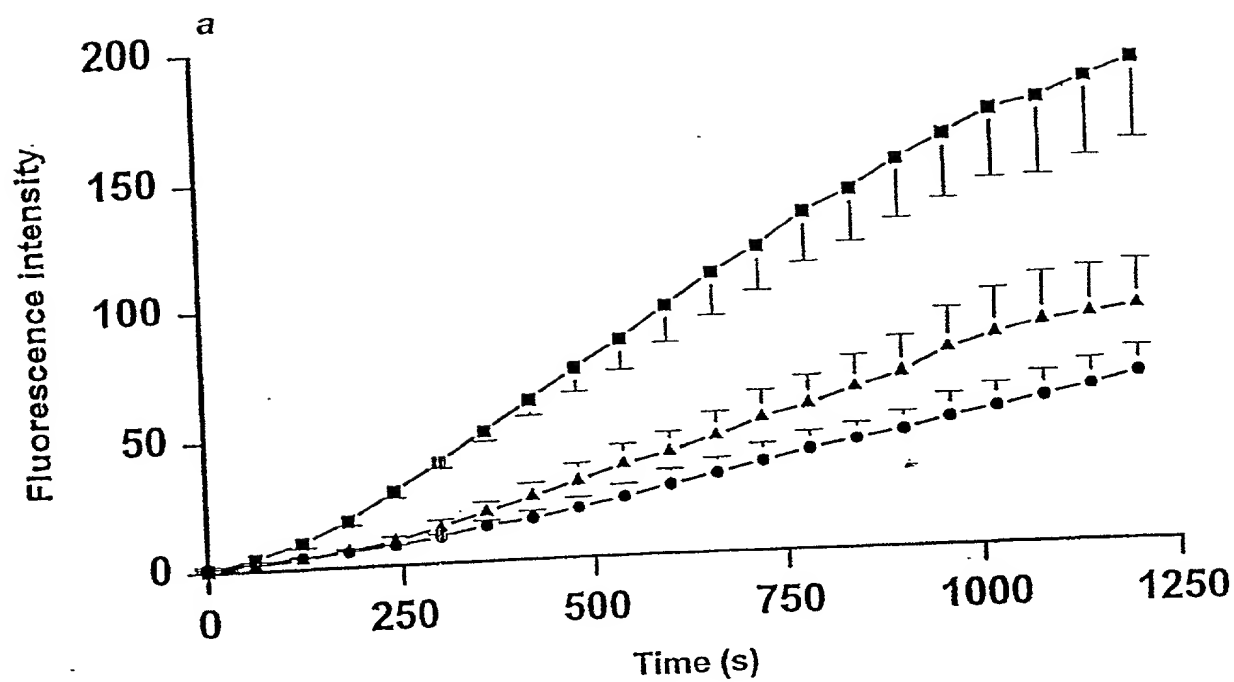


FIG. 12

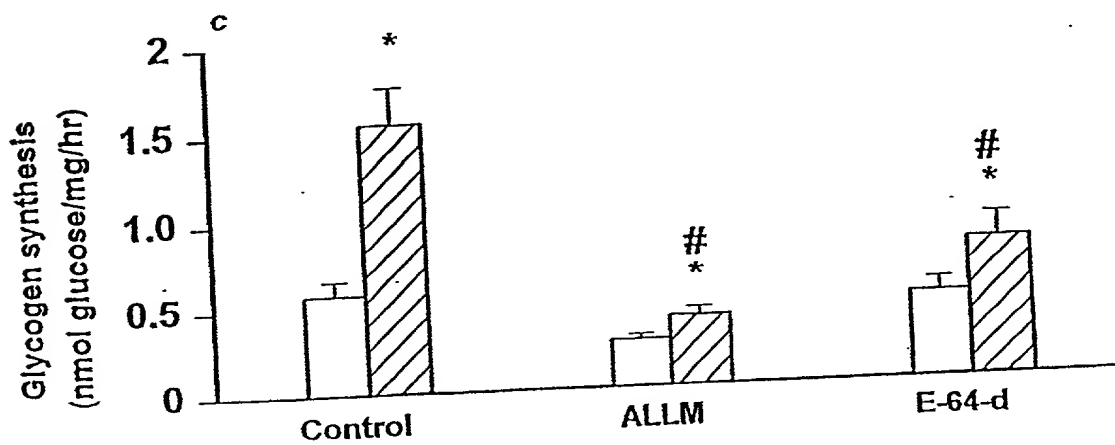
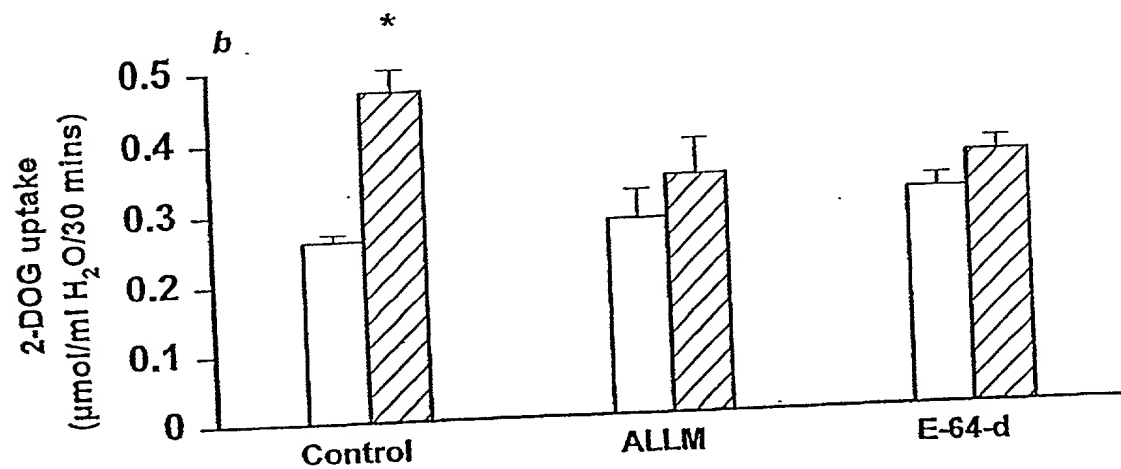
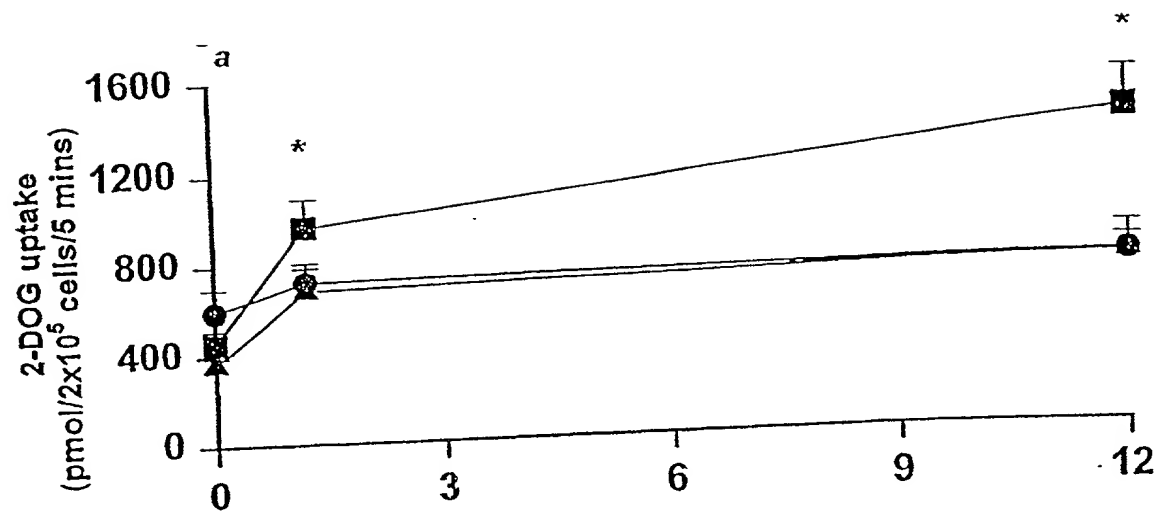
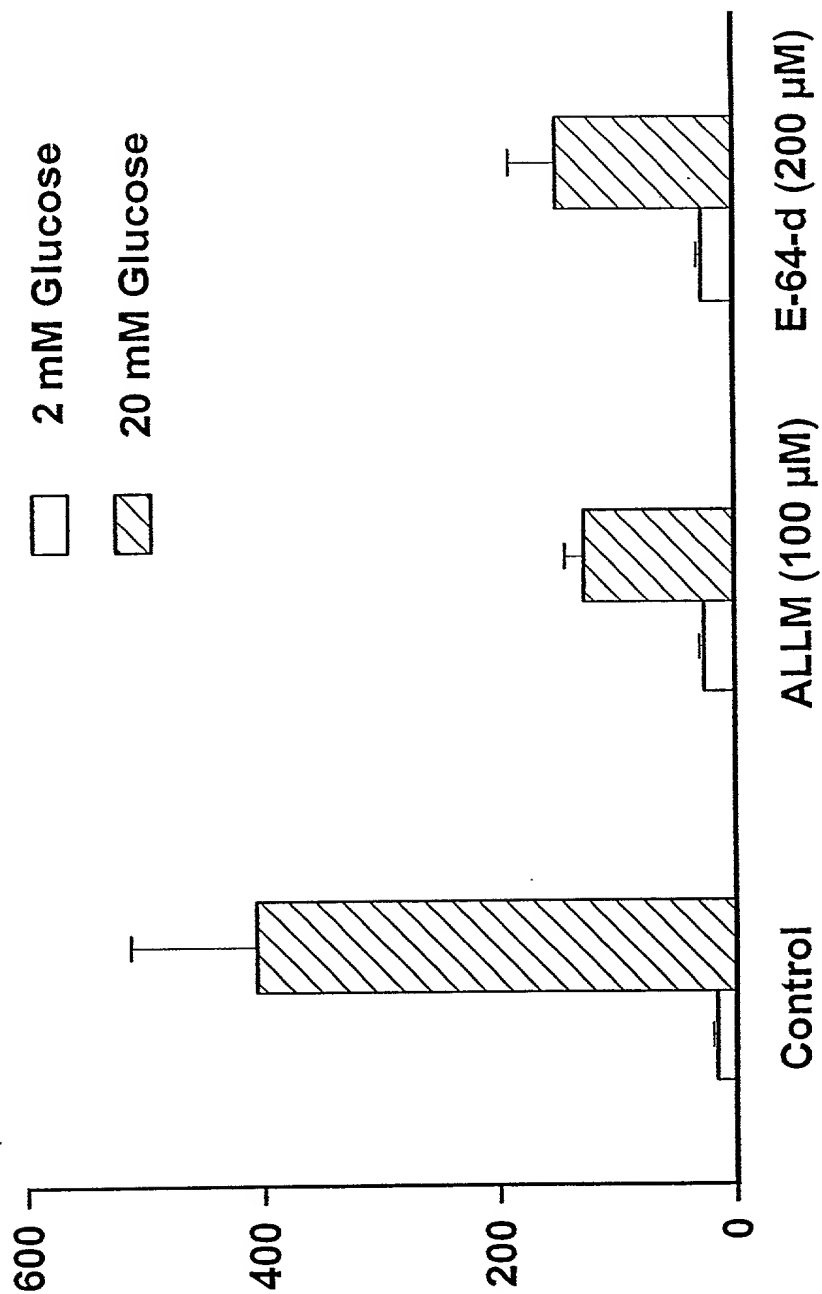
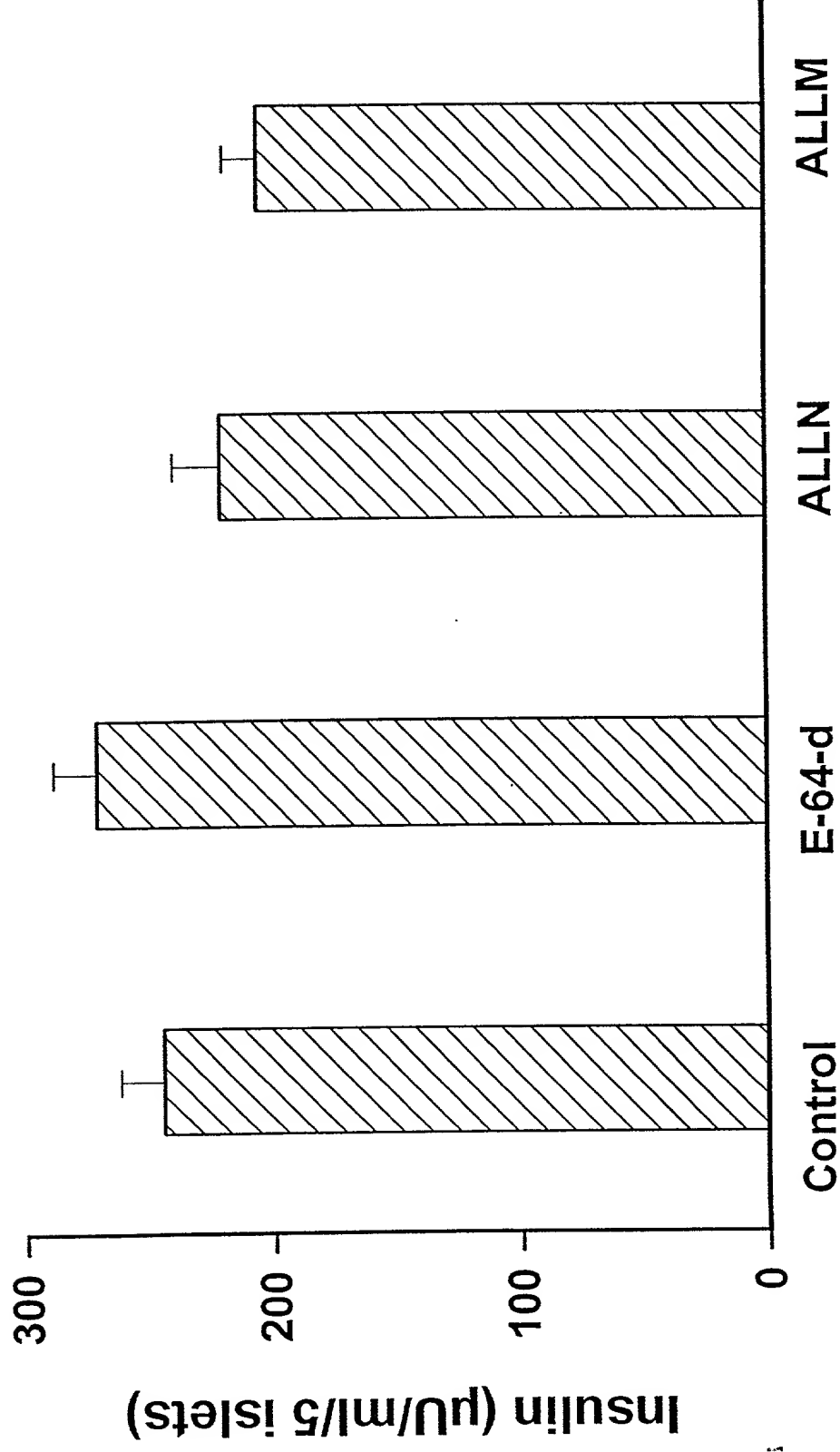


FIG. 13

Fig14 Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion



**Fig 15** Insulin content in 48 hour cultured islets (n=4)



**Fig16.** ALLM dose response in 48 hour treated islets

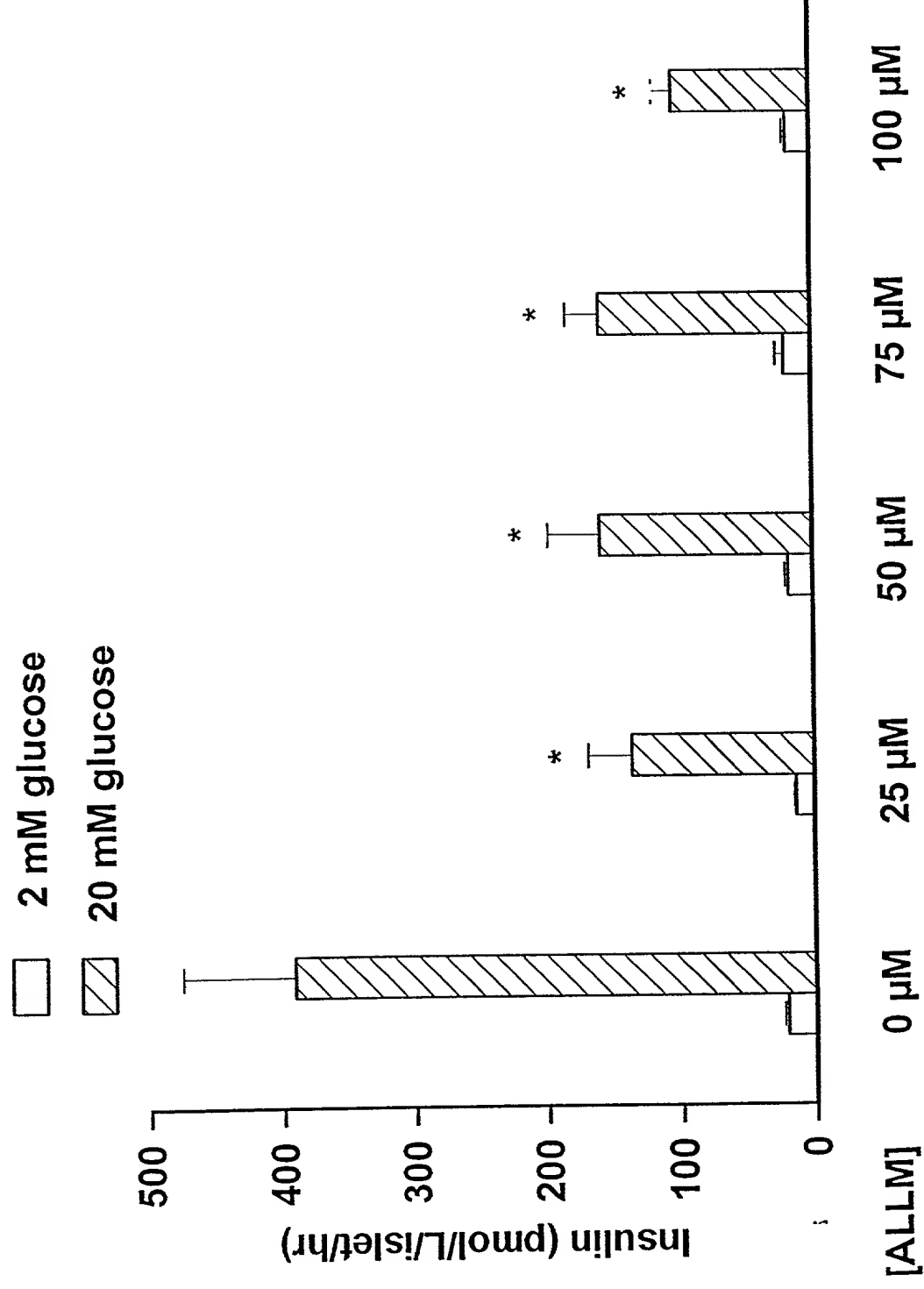
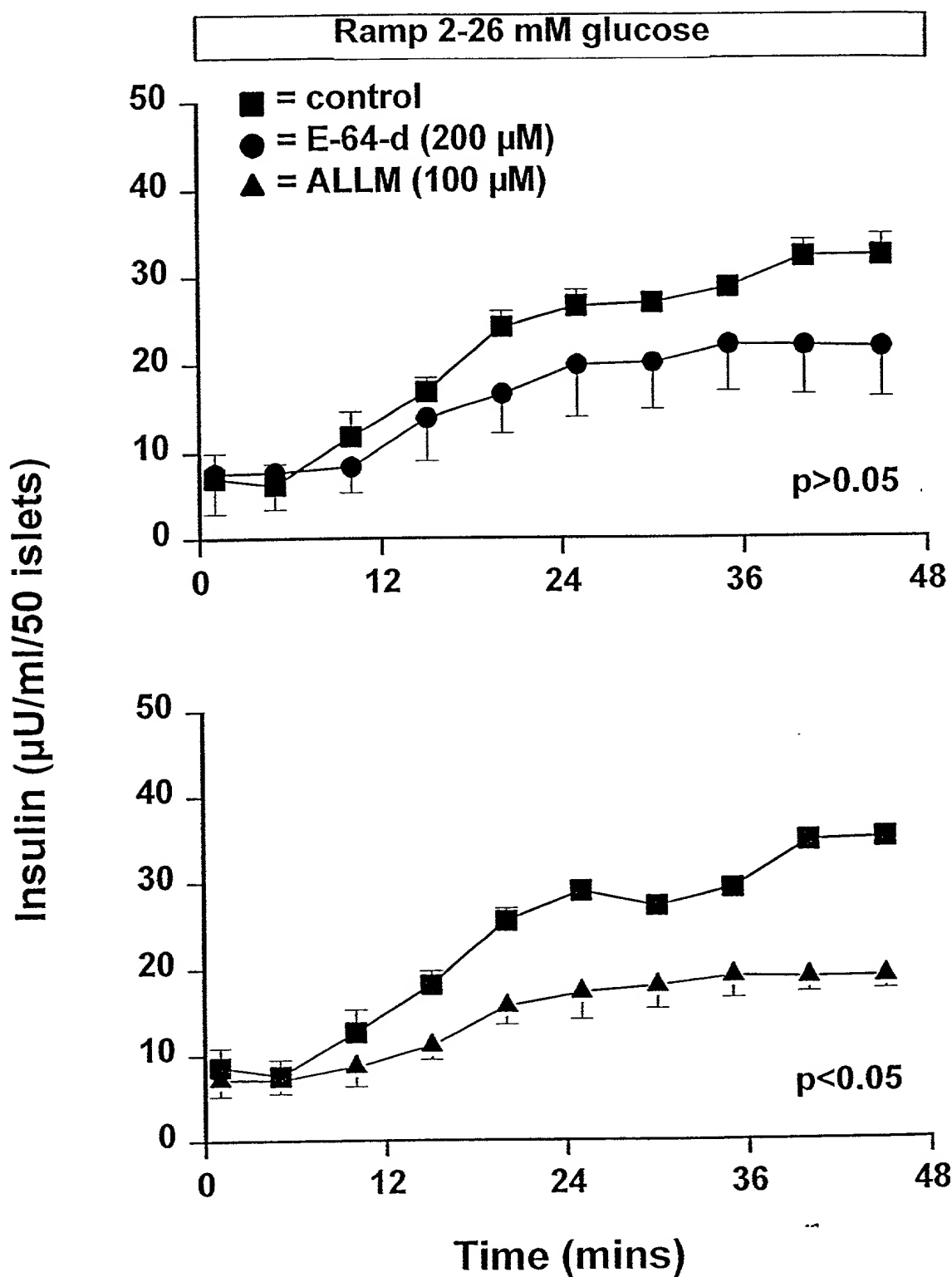




Fig17. Perifusion of 48 hour cultured islets (n=4)



# Insulin secretion in ALLM or E64-d treated mouse islets: Reversal study

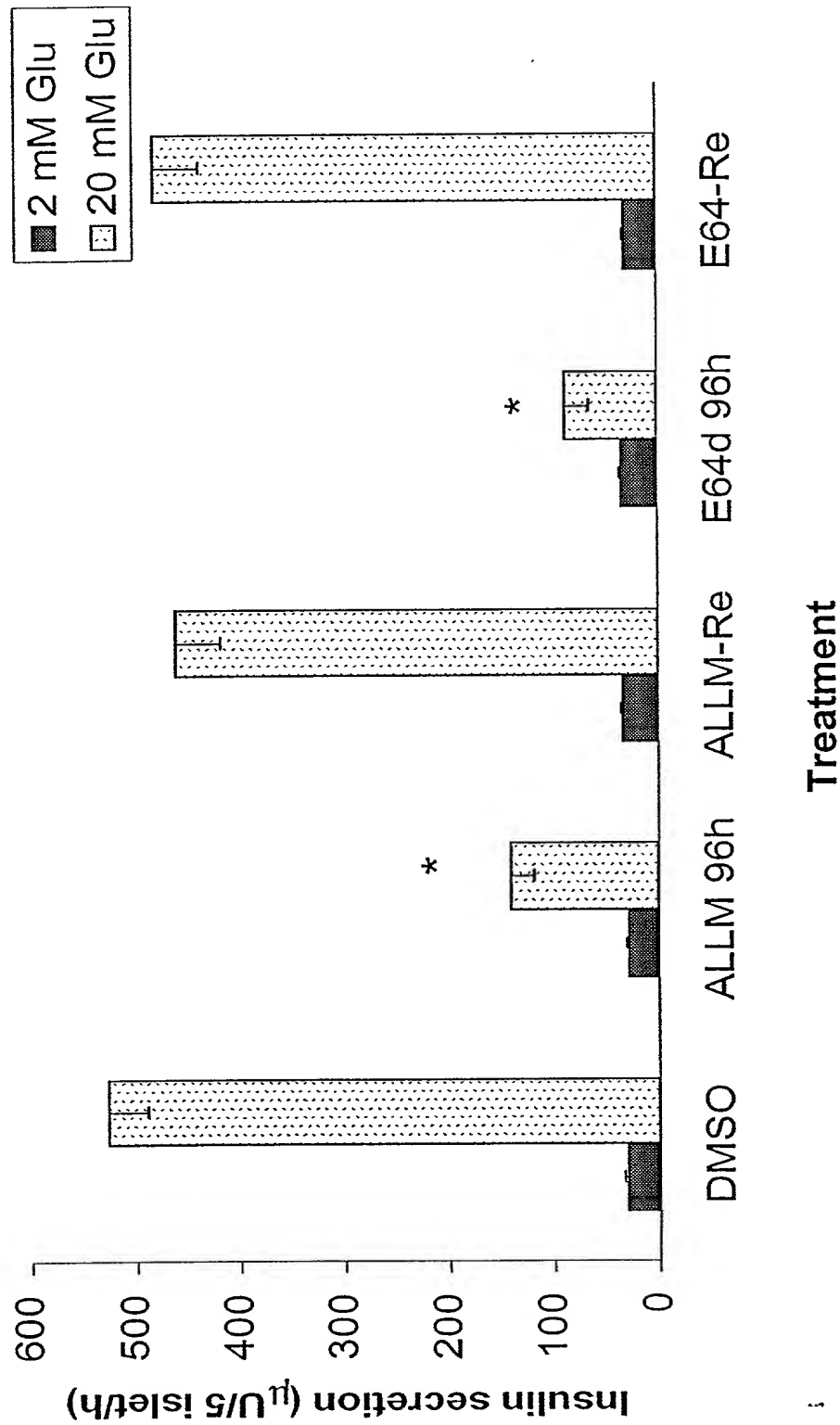
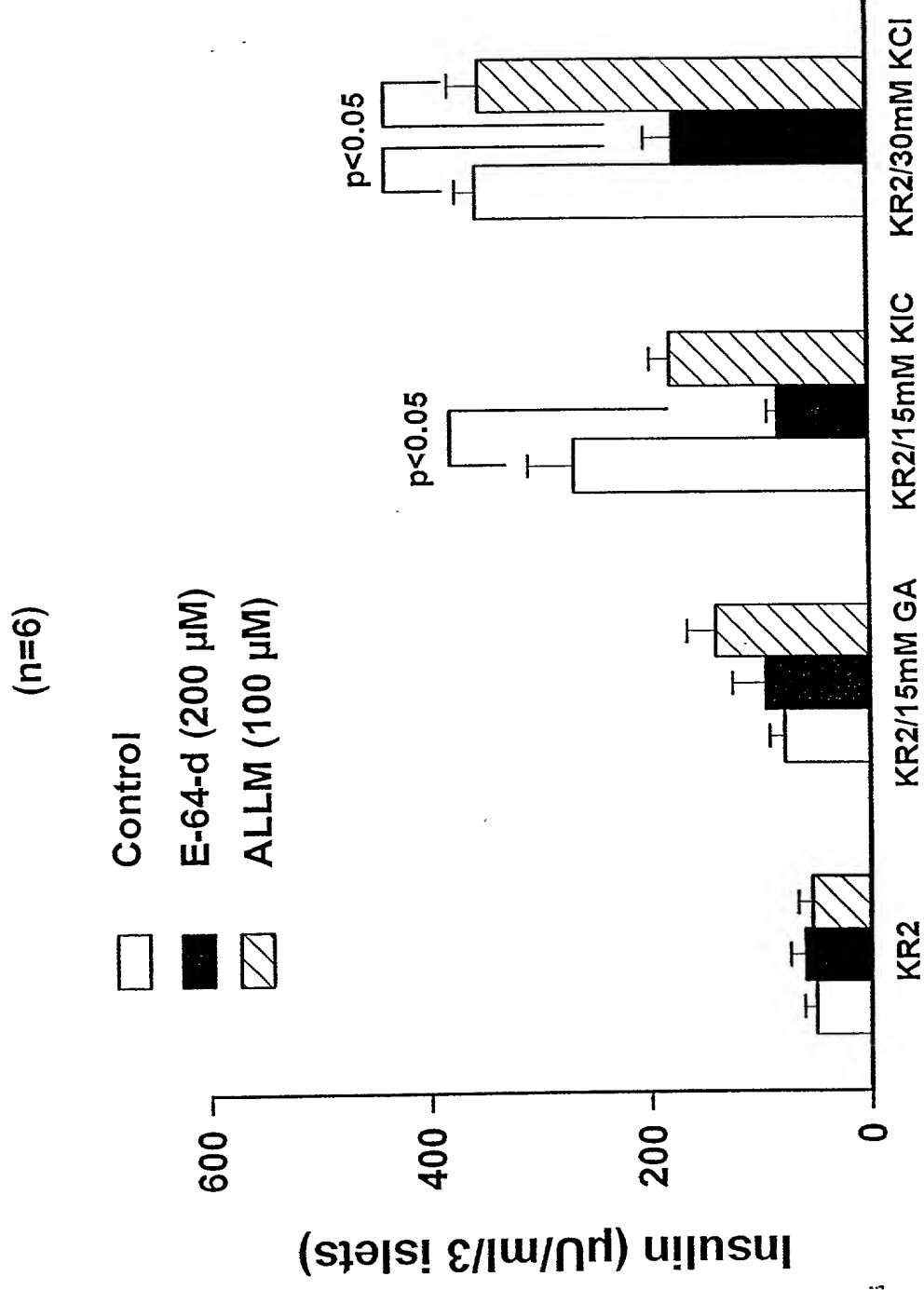


Fig. 18

Fig19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs



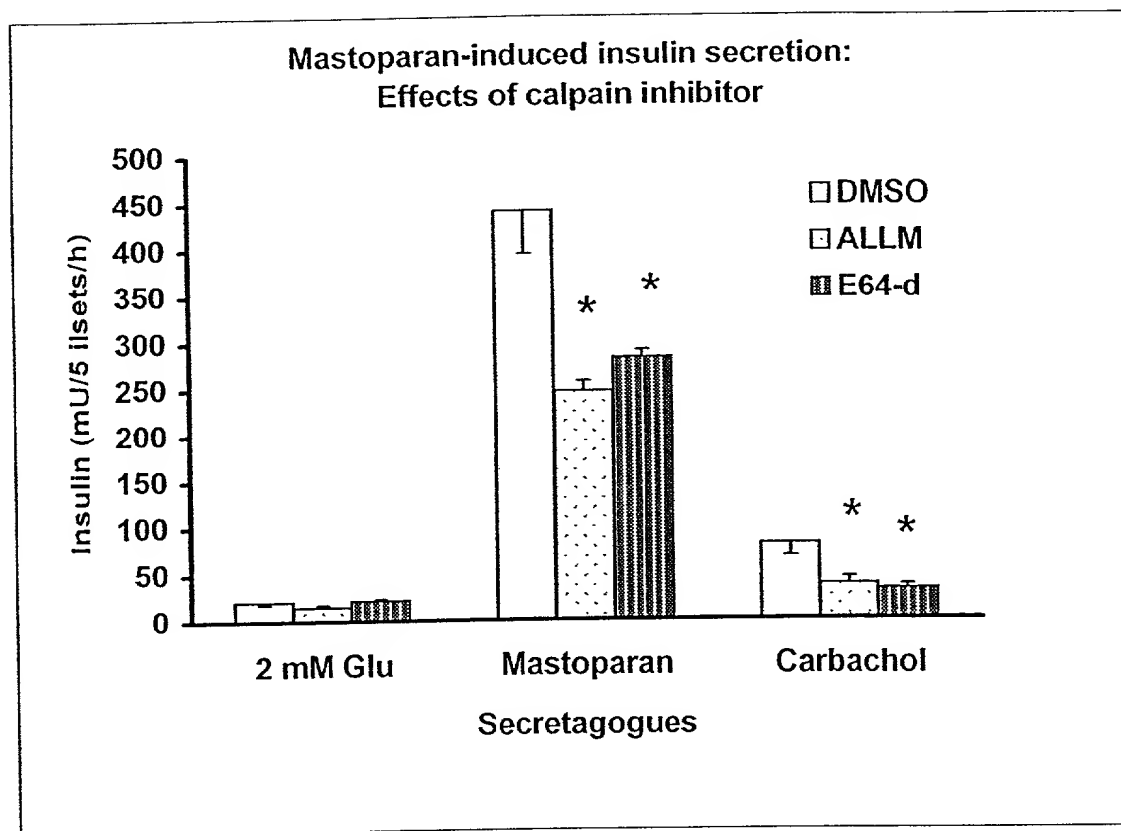
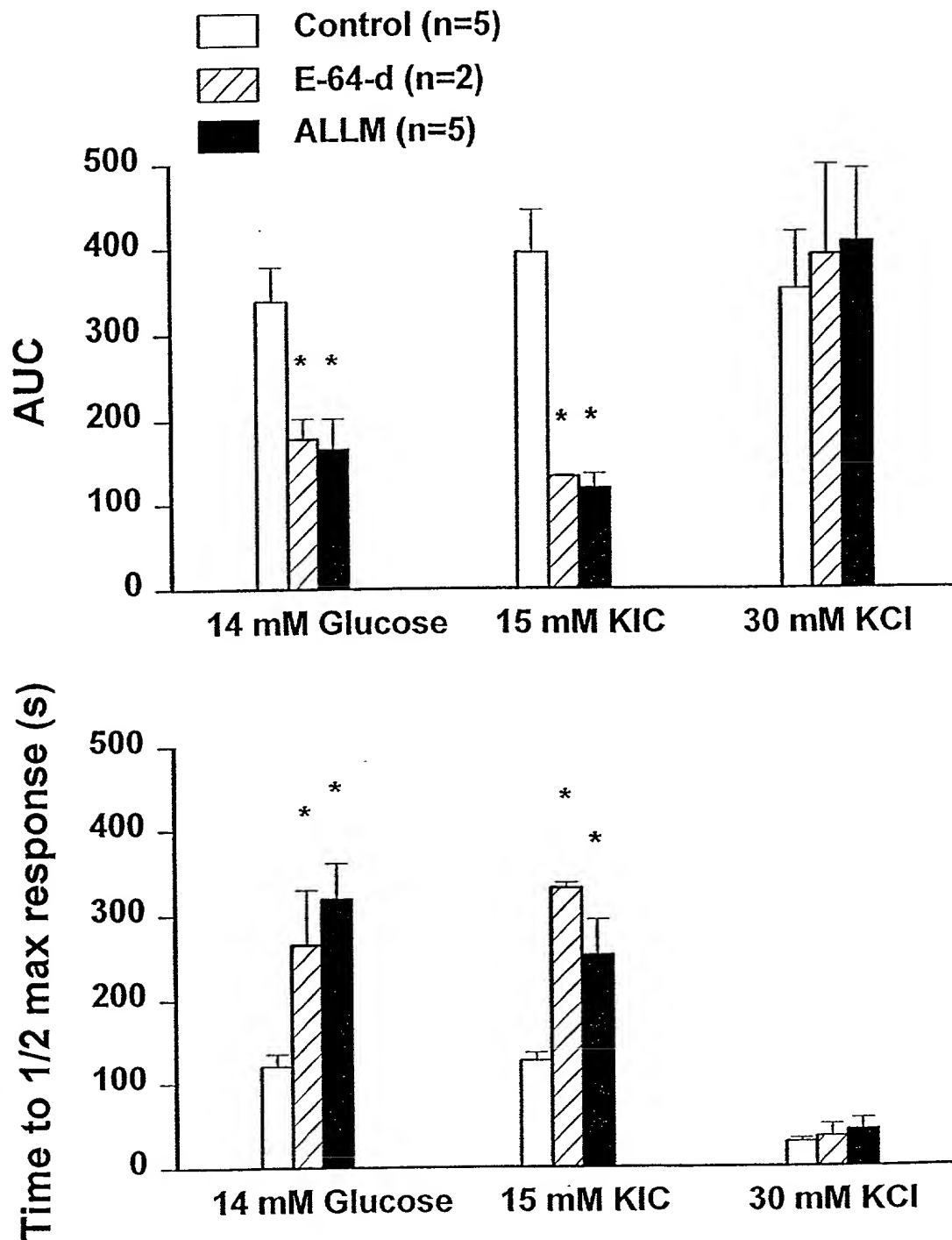


FIG. 20

Fig 21.  $[C^{2+}]_i$  responses to glucose, KIC and KCl



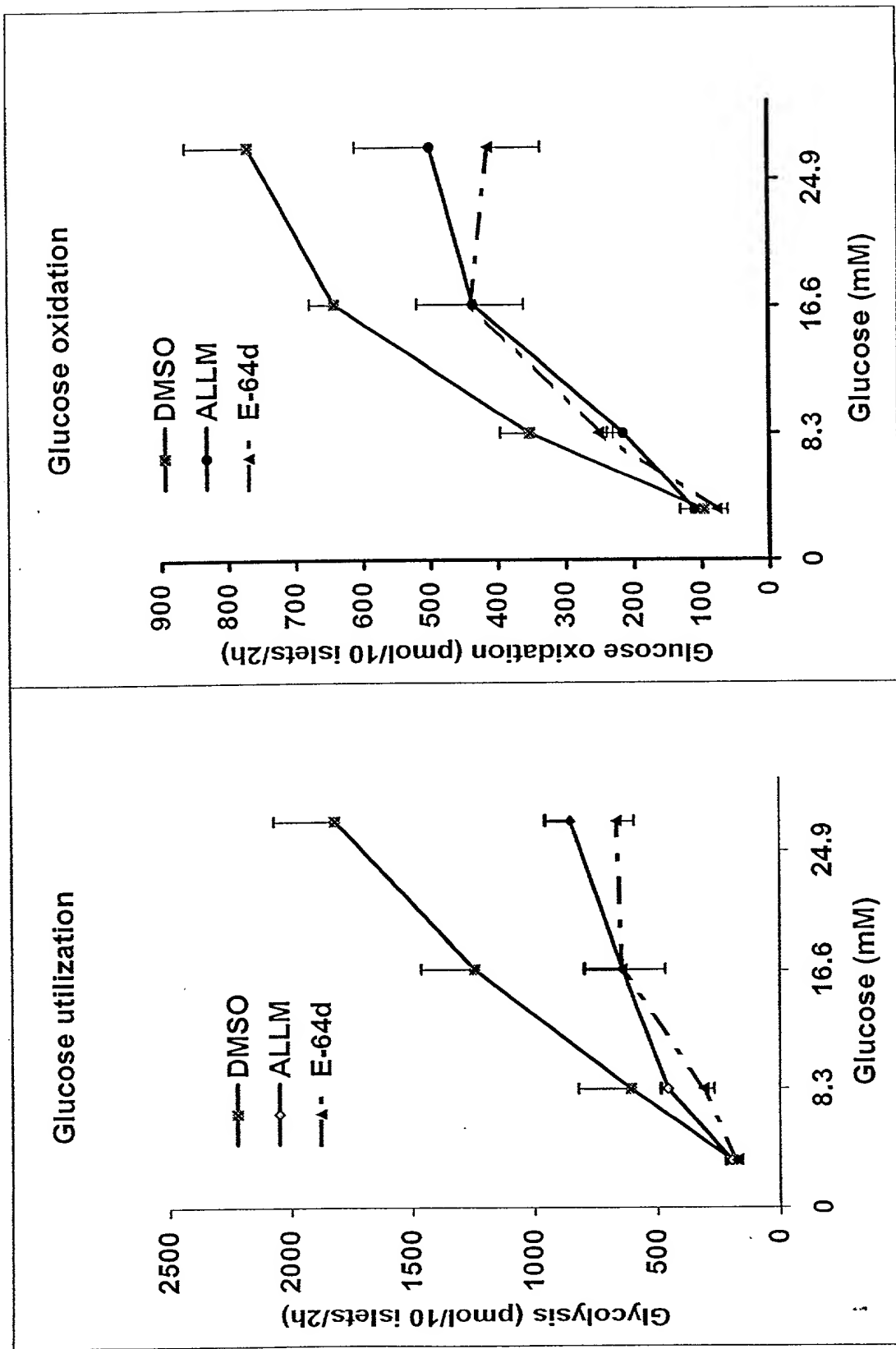
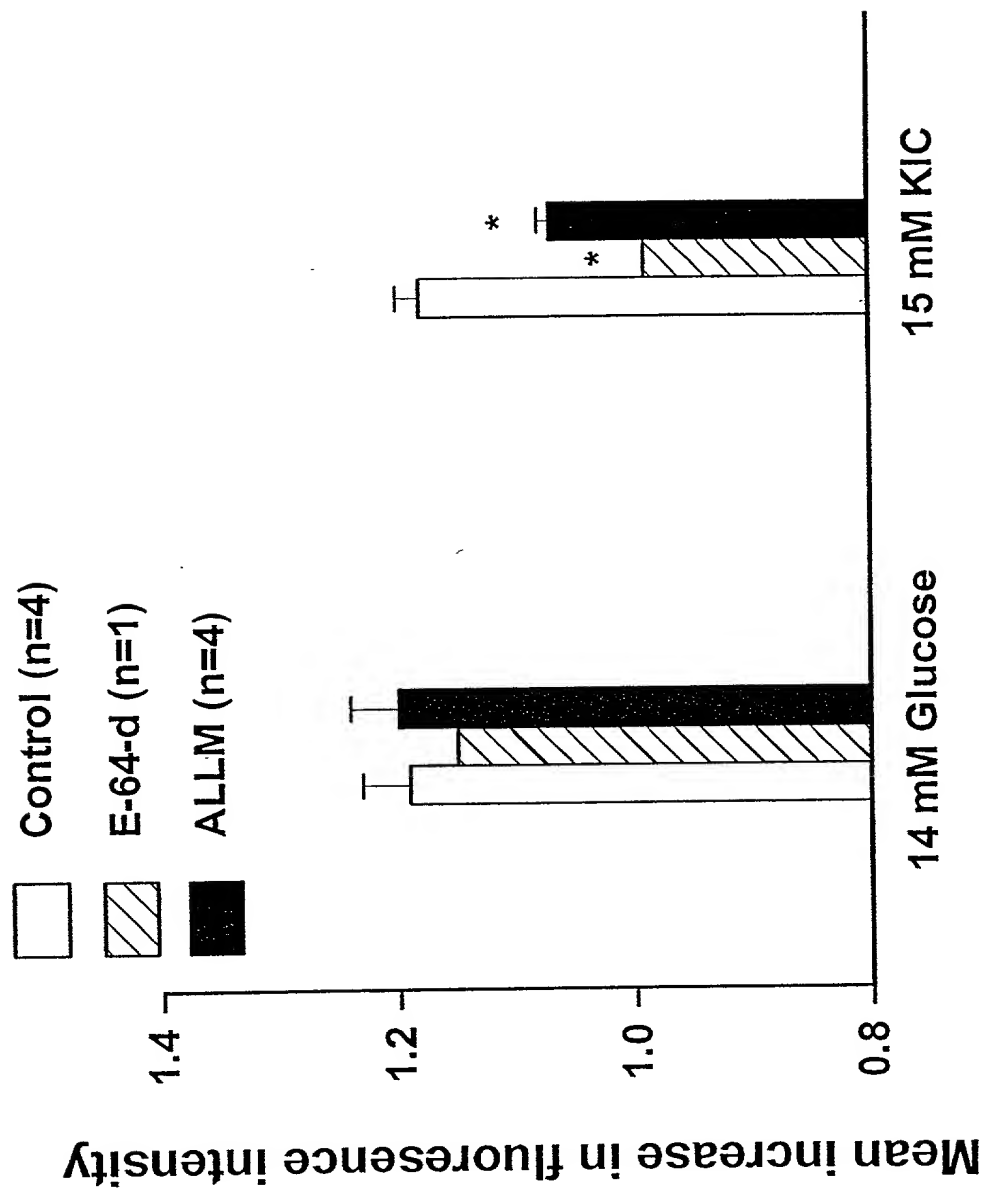


Fig. 22

**Fig 23. NAD(P)H responses to glucose and KIC**

Control

ALLM

100 fF

20 s

Total Secretion (fF)

A vertical number line with tick marks at 0, 50, 100, 150, 200, and 250.

Control

ALLM

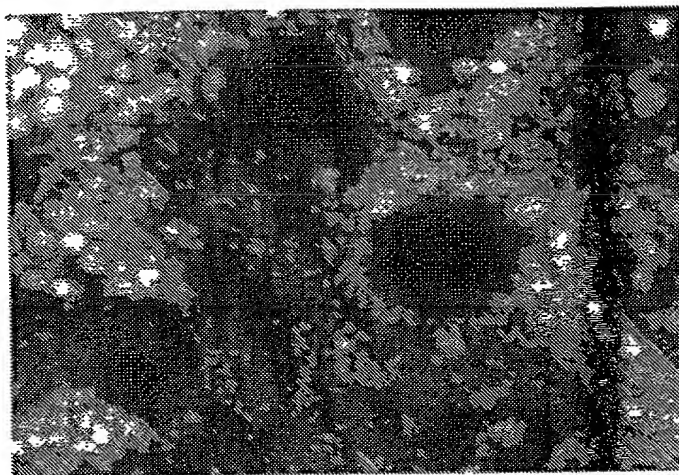
**Fig. 24.** Measurement of membrane capacitance in isolated  $\beta$ -cells



DMSO



E64d



ALLM

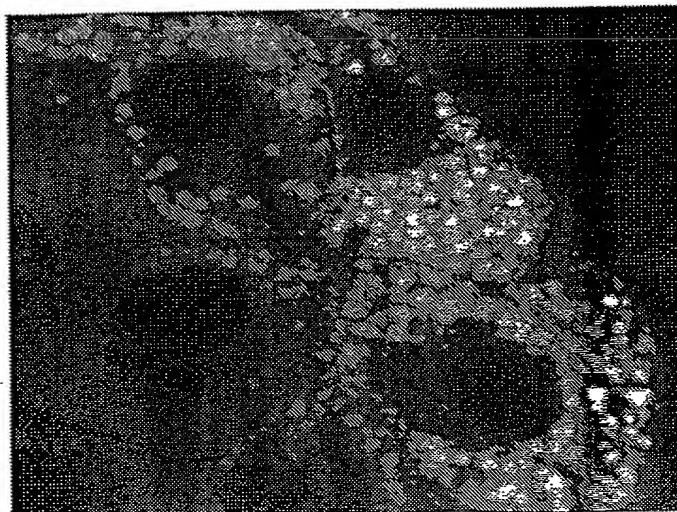


FIG. 25

09768877 "08101  
T02T80" 2889260

20250422093250

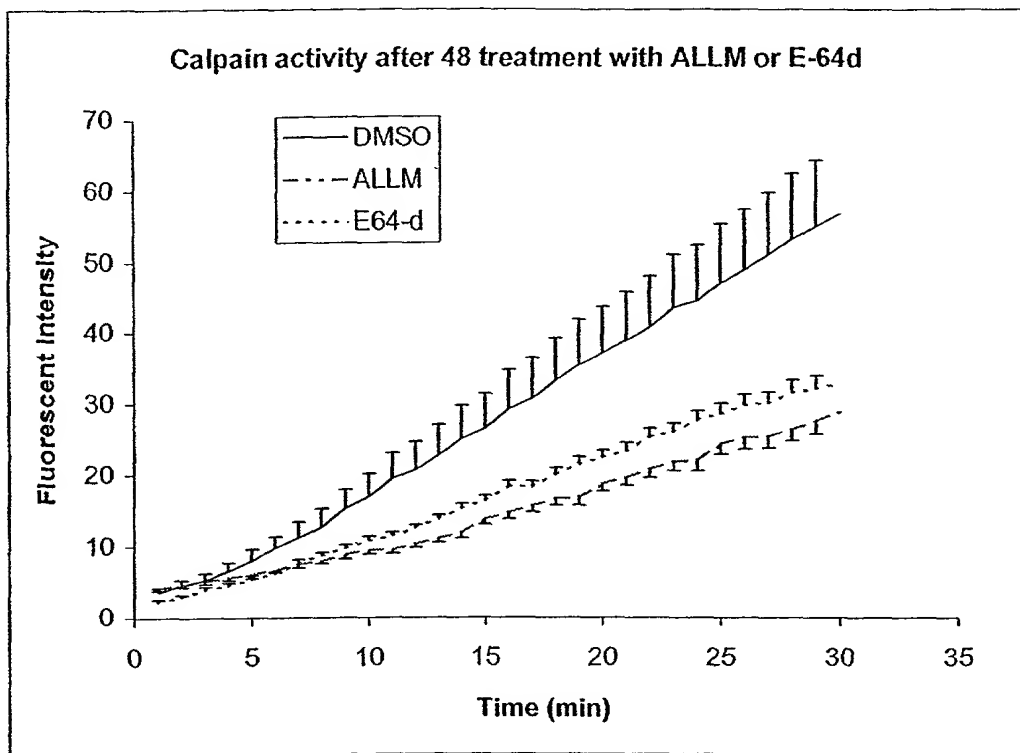


FIG. 26